In vivo imaging and quantification of bacterial infection using a new red fluorescently labeled agent

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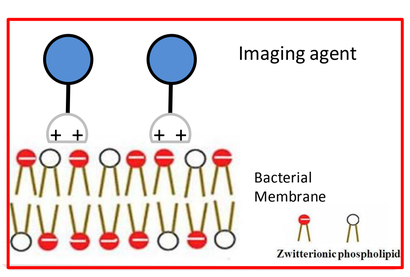
Abstract

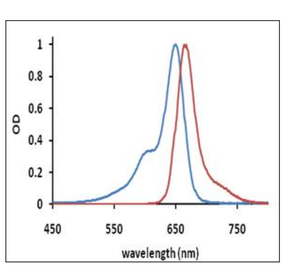
Bacterial infection is a serious and costly clinical issue, so there is significant need for preclinical tools for assessing infection and therapeutic intervention. Rapid and non-invasive imaging technologies and targeted imaging agents would prove invaluable in diagnosing and managing bacterial infections. To this end, we developed a unique type of fluorescent agent that can quickly target bacteria, providing a fluorescent method to quantify bacterial load during infection as early as one hour post-injection. A red cationic agent (BacteriSense™ 645) targeting the negative charge at the surface of bacteria was developed (ex/em 635/656 nm). Specificity of binding to negative-charged molecules present on the bacterial membrane and cell envelope was tested using various glycolipids and lipopolysaccharide (LPS) bound to 96-well plates in the presence or absence of a competing positively-charged molecule. BacteriSense 645 was able to effectively bind to negatively (phosphatidylserine, phosphatidylethanolamine, LPS and teichoic acid) but not to a neutral (phosphatidylethanolamine) charged surfaces, and a positively-charged competitor was able to significantly reduce binding. These results suggested that important negatively-charged components in both Gram-negative and Gram-positive bacteria were recognized. This was confirmed by in vitro binding of BacteriSense 645 to Gram⁻ *Escherichia coli* and Gram⁺ *Staphylococcus epidermidis* and assessment by fluorescence microscopy and flow cytometry. BacteriSense 645 was found to bind to *E.coli* more efficiently than *S. epidermidis* (4 fold) and not to mouse skeletal muscle cells serving as a negative control. In vivo pharmacokinetic profiles in CD-1 and infected SKH-1 mice showed a plasma half life of 15-45 min, depending on level of infectious burden, providing rapid clearance from circulation. Sensitivity of detection was assessed in SKH-1 E mice infected intramuscularly in the thigh with 10^5 to 10^8 CFUs of *E. coli* and injected intravenously 24h later with 5 nmoles of BacteriSense 645. Bacterial loads as low as 10^5 were detectable in mice imaged using the fluorescence tomography system (FMT2500), and this imaging agent was highly unique in its ability to provide optimal imaging just 1h following injection with a tissue half life of 3h and washout of the signal (blood and tissue) by 24 h, allowing daily repeat imaging in longitudinal infection models. Similar imaging results were obtained with *S. epidermidis* infected mice, supporting this agent's utility in Gram-positive bacterial infections as well. More importantly, BacteriSense 645 was able to distinguish E. coli infection from LPS injected simultaneously in the contralateral thigh (106 +/- 31 vs 21 +/- 10 pmoles respectively by tomography, p=0.029; 0.051 +/- 0.005 vs 0.035+/-0.001 mean counts/energy by reflectance, p=0.0113). Taken together, these results highlight the value of BacteriSense 645 in preclinical drug development studies.

1 BacteriSense 645 Characterization

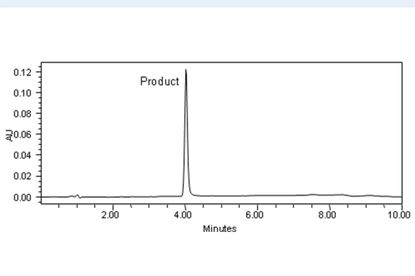
A. Phycochemical properties



B. Abs/Em properties

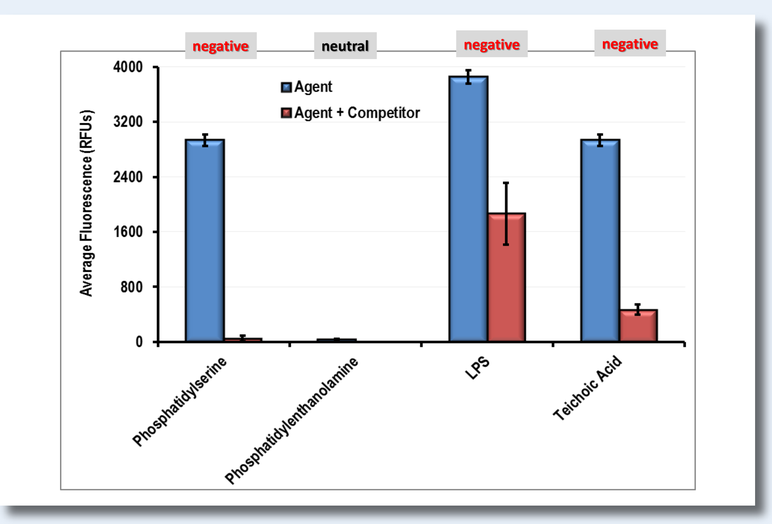


C. Purity



A. The red cationic agent, BacteriSense 645, targets the negative charge at the surface of bacteria **B.** The agent has an absorption peak at 635 nm and emission peak at 656 nm in 1x PBS. **C.** Purity was shown to be >98% at 640 nm as analyzed by RPLC-UV-MS.

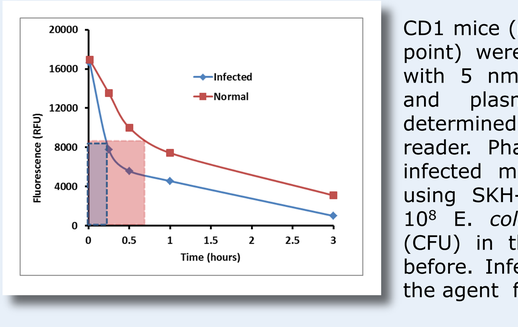
D. Specificity



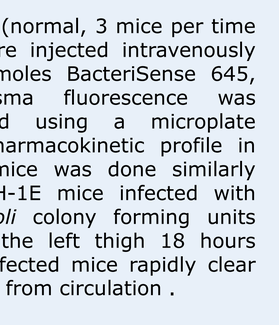
D. Bacterisense 645 was able to effectively bind to negatively charged molecules bound to 96-well polysorb FluoroNunc plates but not neutral ones. Binding of Bacterisense 645 to negatively-charges molecules was significantly reduced in the presence of a 100 fold molar excess of a competitor, showing the specificity of the agent.

2 Cell Labeling

A. Binding to Bacteria



B. Specificity



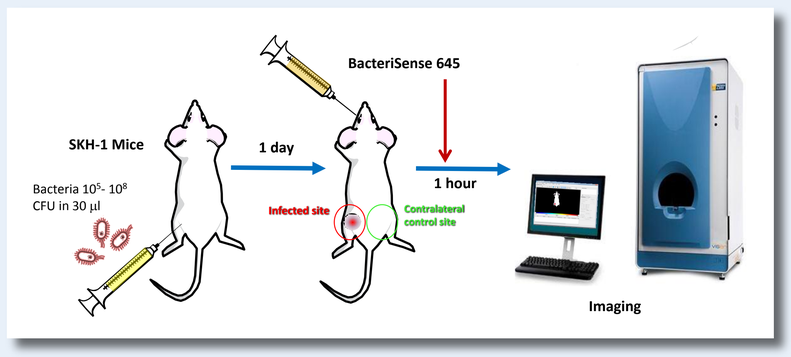
A. Escherichia *coli* and Staphylococcus *epidermidis* were labeled with 10 μM BacteriSense 645 in TES buffer for 1 hour at RT. Bacteria were visualized by fluorescence microscopy (Zeiss Axioscop and Volocity software) and fluorescence quantified by FLOW cytometry (BD cytometer). The agent (red) binds both strains of bacteria with different efficiency. **B.** Mouse muscle cells and E. *coli* were labeled with BacteriSense 645 (10 μM) in TES buffer for 15 min at RT. Cells were visualized by fluorescence microscopy. Muscle cells were counterstained with DAPI nuclear stain (Blue). Muscle cells did not bind the agent. This experiment validates the specificity of BacteriSense 645 towards bacterial cells.

3 Pharmacokinetics

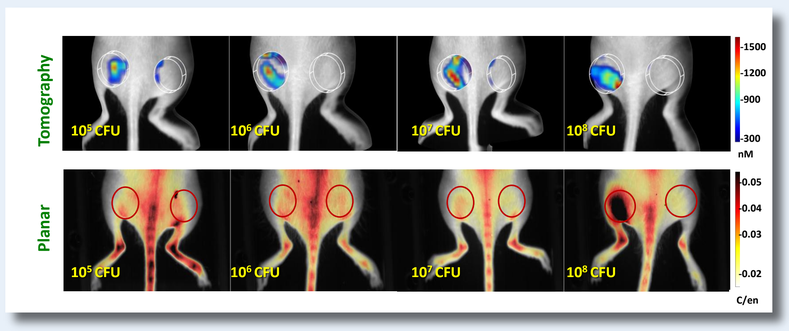
CD1 mice (normal, 3 mice per time point) were injected intravenously with 5 nmoles BacteriSense 645, and plasma fluorescence was determined using a microplate reader. Pharmacokinetic profile in infected mice was done similarly using SKH-1E mice infected with 10^8 E. *coli* colony forming units (CFU) in the left thigh 18 hours before. Infected mice rapidly clear the agent from circulation .

4 Sensitivity of In Vivo Imaging

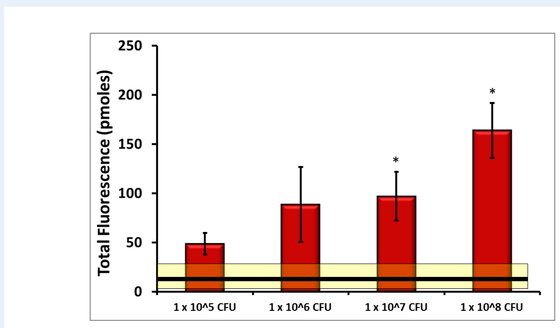
A. Schema of the Infection Model



B. In Vivo Imaging



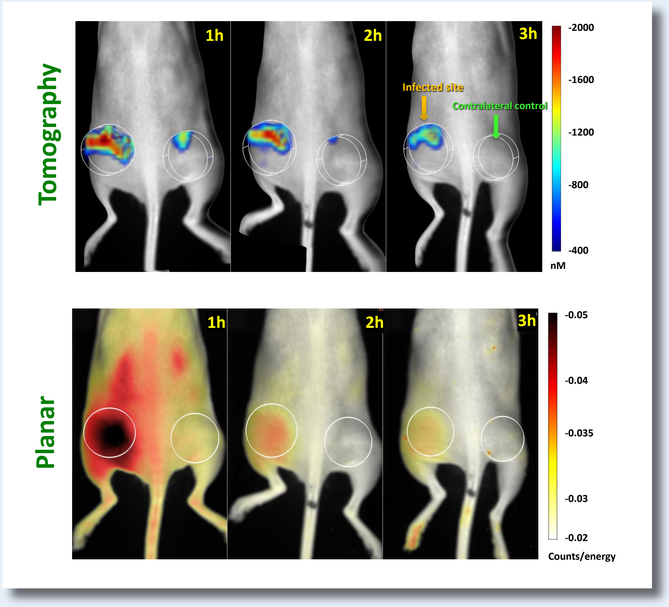
A. SKH-1E mice were injected intramuscularly in the left flank with 10^5-10^8 E. *coli* CFU. Eighteen hours later, mice received an intravenous injection of BacteriSense 645 (5 nmoles) and imaged 1 hour later by FMT2500 in both tomographic and planar modes. **B.** Fluorescence 3D tomography and planar 2D images of representative. The color bar represents fluorescence concentration in nM or fluorescence brightness in counts/energy).



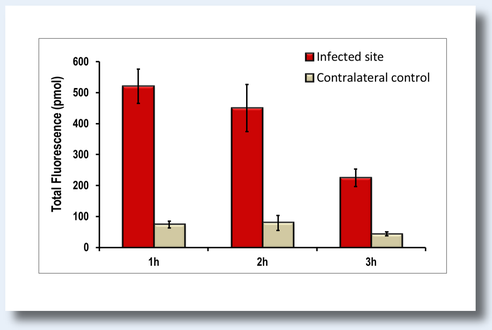
C. Quantification of fluorescence signal emanating from the infected site. The graph of total fluorescence (in pmoles) across different experimental groups reveals that as few as 10^5 CFU can be detected above background (contralateral thigh, shown as a yellow bar).

5 Kinetics of In Vivo Imaging

A. In Vivo Imaging

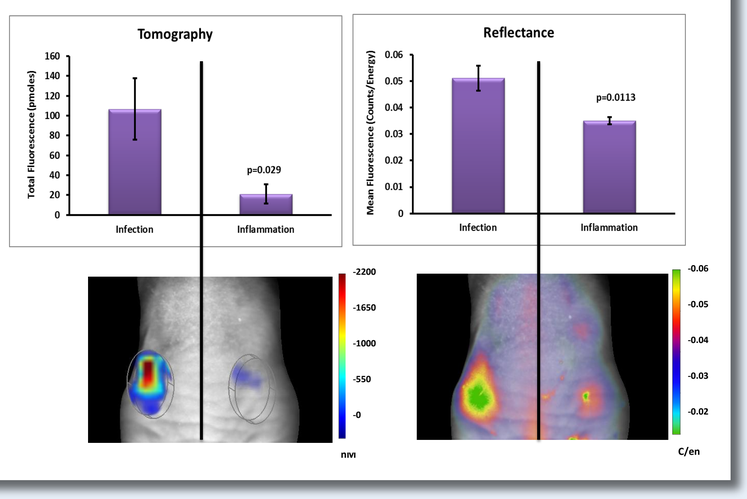


C. Quantification



SKH-1E mice were infected with 10^8 CFU S. *epidermidis* in the left thigh muscle. Eighteen hours later, mice were injected intravenously with BacteriSense 645 and imaged at 1, 2 and 3h. **A.** Fluorescence tomography and planar images of representative mice are shown. **B.** Quantification of fluorescence signal shows a significant difference in the amount of signal (in pmoles) in infected *versus* control sites. At 3h, only half of the fluorescence is still present in the infected site. Rapid tissue clearance allows for repeated dosing and imaging.

6 Infection versus Inflammation



Mice were infected with E. *coli* in the left thigh muscle and with lipopolysaccharide (25 μg LPS) in the right thigh muscle. Seventeen hours later, mice were injected with BacteriSense 645 and imaged at 1h. Tomographic and planar images of a representative mouse are shown. Quantification of fluorescence signal by tomography and reflectance shows that BacteriSense 645 can discriminate between infection and inflammation (p=0.026).

Summary

We have developed a far red fluorescent bacterial imaging agent (BacteriSense 645) to label both Gram⁺ and Gram⁻ bacteria via binding to negatively-charged lipids and polysaccharides unique to the bacterial membrane. Standard techniques of assessing progression of bacterial infections in vivo rely on terminal collection of infected tissue for histology or culture. In vivo imaging using BacteriSense 645, however, provided a noninvasive and faster alternative for the accurate detection and quantification of infectious foci as soon as 1h post injection. Further, the rapid clearance of the agent from both blood and infected tissue allowed daily imaging of changes in the infectious burden. Our results highlight the value of BacteriSense 645 in preclinical drug development studies and will hopefully help foster the development of new therapeutic and surgical strategies.

7 References

W. Matthew Leevy, Nathan Serazin, and Bradley D. Smith. **Optical** Imaging of Bacterial Infection Models**.** Drug Discov Today Dis Models. 2007 Fall; 4(3): 91–97.

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